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(54) Title: ORAL CREATINE SUPPLEMENTATION FOR TREATING OR PREVENTING MUSCLE DISUSE SYNDROME

(57) Abstract

The present invention relates to the use of a creatine compound, in particular creatine or a creatine analogue for the manufacture of a therapeutic preparation for the prevention or treatment of muscle disuse syndrome in a subject. The invention further relates to a therapeutic preparation for treating or preventing muscle disuse syndrome, comprising a suitable carrier, diluent or excipient and an effective amount of one or more creatine compounds.

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# ORAL CREATINE SUPPLEMENTATION FOR TREATING OR PREVENTING MUSCLE DISUSE SYNDROME

The present invention relates to the prevention and treatment of muscle disuse syndrome.

Muscle disuse syndrome is defined as the reversible deterioration of structural, functional, metabolic and neuromotor properties of skeletal muscle tissue as a result of reduced mechanical loading. The degenerative symptoms typical to the muscle disuse syndrome include muscle atrophy (decreased muscle volume), reduced maximal force, reduced maximal power, increased muscle relaxation time, premature muscle fatigue, reduced muscle energy stores, reduced muscle blood flow, reduced insulin sensitivity, and impaired motor control.

The muscle disuse syndrome may occur in any skeletal muscle subject to reduced mechanical loading due to whatever cause. Thus, mechanical unloading may occur as a result of any disease condition, ageing, physical and/or mental handicap, forced bed rest, or any other condition associated with a reduced level of physical activity. Increased mechanical loading of the muscle, either by muscle rehabilitation training or by resumption of a normal level of physical activity, reverses the muscle disuse syndrome.

One example of a condition in which muscle disuse syndrome may occur is immobilization, for example when a broken extremity like a leg or an arm is immobilized in a cast for some weeks, or when a patient is forced to bed rest due to disease. The atrophy is then usually clearly visible by a slimming of the leg(s) or arm(s).

It should be noted that the muscle disuse syndrome here described does not lead to the death of muscle cells. The atrophy that is found as an effect of the syndrome leads solely to a decrease in cell volume and functional capacity. However, there is no

irreversible destruction of the cells, as is the case in muscle dystrophy.

It is the object of the invention to provide a therapeutic preparation for treating or preventing muscle disuse syndrome in a subject.

According to the invention it has now been found that a relatively low dosage of one or more creatine compounds can reduce the effects of muscle disuse syndrome. Thus, by administering to a subject who is under the risk of developing or suffering from the muscle disuse syndrome, a suitable amount of one or more creatine compounds the effects of the syndrome can be reduced or even avoided, and the rehabilitation of the syndrome can be enhanced.

The use of creatine for various purposes has been described in the literature. However, neither of these disclosures are related to the actual treatment of muscle disuse syndrome or of muscles that are in principle healthy. Although the muscle atrophy to which this invention is directed are decreased in cell volume and functional capacity, they are not diseased or dying like in muscle dystrophy.

Mahanna et al. (Exp. Neurol. (1980), 68(1), 114-121) disclose the effect of ß-guanidinopropionic acid (ß-GPA) on skeletal muscle for evaluating the need for creatine and phosphocreatine in the maintenance of muscle fiber size. However, ß-GPA not only leads to muscle creatine depletion, but also the ATP concentration in the muscles studied is markedly reduced. Conversely, in healthy muscle cells ATP concentration is normal, even during the most severe conditions of disuse. The decrease of muscle ATP content upon ß-GPA administration is a typical symptom of the toxic effects of ß-GPA on muscle, that in turn cause muscular adaptations that are markedly different from the normal physiological adaptations of skeletal muscles to mechanical loading (training) and unloading (disuse), or creatine administration. In fact, animal studies using ß-GPA administration may possibly

serve as an experimental model of very severe conditions of pathologic muscle dystrophy, but not of the symptoms of the muscle disuse syndrome as described herein. The toxic effects of GPA, indeed, induce cellular adaptations that to a large degree are different from the reversible physiological muscular adaptations during the muscle disuse syndrome (Laskowski B et al., Metabolism 30, 1080-1085, 1981; Moerland TS et al., American Journal of Physiology 257, C810-C816, 1989; De Saedeleer M & Marechal G, Pflügers Archives European Journal of Physiology 402, 185-189, 1984; Levine S et al, American Journal of Physiology 271, C1480-C1486, 1996). In fact, GPA-treatment is used as a model for muscle dystrophy. Results obtained with diseased muscle cells are not indicative for healthy muscle cells.

Wyss et al. (Medical Hypotheses (1998), 51(4), 333-336) is also concerned with oral creatine supplementation in muscle disease, such as Duchenne muscle dystrophy, for alleviating the clinical symptoms.

WO98/00148 relates to drug preparations that contain creatine and at least one calcium, magnesium, manganese or zinc salt to reduce the creatine dose.

Oral creatine supplementation of healthy individuals, in particular athletes, has been shown to improve performance during short maximal exercise bouts. Although long-term creatine supplementation has rapidly developed as a standard ergogenic practice in athletes, there was initially no scientific evidence that this practice could be in fact beneficial. It has been suggested that the observed increase of fat free mass occurring as a result of creatine supplementation was solely due to body water accumulation and not to muscle hypertrophy.

The use of creatine according to the invention for the treatment of muscle disuse syndrome, which in principle involves healthy muscles that have a decreased cell volume and functional capacity, but are in fact

still intact, cannot be derived from these prior art documents.

The treatment of the invention can be a preventive treatment when the therapeutic preparation is administered to the subject from the onset of the risk to develop the syndrome. Also, the preparation can be given to patients already suffering from the syndrome.

For the effective treatment or prevention of muscle disuse syndrome, the creatine compound is to be administered on a daily basis, or on an intermittent basis in a total daily amount of between 0.5 and 10 g, preferably between 1 and 5 g, such as about 2.5 g, per day. This amount can also be given in more than one portion over the day. The one or more creatine compounds can be combined with suitable excipients, diluents, carriers etc. to obtain a dosage form for administration. Suitable dosage forms are drinks, tablets, capsules, powders, sweets or any nutritional supplement or nutrient containing added creatine.

Surprisingly, it has further been found that the administration of one or more creatine compounds can lead to improved glucose tolerance, an increased insulin sensitivity of the muscle, an increase in muscle capillarisation and an enhanced muscle relaxation. The latter not only reduces relaxation-dependent energy consumption in muscles during exercise, but at the same time may conceivably improve muscle coordination by reducing the amount of co-contraction activity between agonist and antagonist muscles.

This led the inventors to the additional finding that creatine treatment can also be used to treat muscle disuse syndrome in elderly. Elderly have a reduced mechanical loading due to the fact that they are not as mobile as younger people. Therefore, the muscle disuse syndrome is intrinsic to aging.

Muscle disuse syndrome may also be caused by chronic fatigue syndrome. Creatine supplementation of the invention can also be used to treat the muscle disuse

syndrome that is the result of the chronic fatigue syndrome.

The term "therapeutical preparation" is used in this application to encompass both preparations for treatment, i.e. drugs, and nutritional supplements for example in the form of food stuffs in liquid or solid form that contain additional creatine. The therapeutical preparation may be used alone or in combination with a physical rehabilitation programme. The latter is, however, not essential for obtaining the desired effect.

The present invention will be further illustrated in the examples that follow. In the examples reference is made to the following figures:

**Figure 1**: Effect of oral creatine intake on m. quadriceps cross-sectional area during immobilization and rehabilitation. Values are mean ± S.E.M. of 10 observations and represent the change of muscle cross-sectional area (CSA) compared with baseline value, which was set equal to zero. S.E.M.'s of some data points are omitted for clarity of the figure. A cast first immobilized the right leg (□,■) during a period of 2 weeks, while the other leg served as a control leg (○,●). Thereafter subjects participated in a 10 weeks rehabilitation program for the knee extensors of the immobilized leg. Subjects ingested either supplementary creatine monohydrate (filled symbols) or placebo (open symbols). * Refers to a significant treatment-effect compared with placebo value in the corresponding leg, $p<0.05$.

**Figure 2**: Effect of oral creatine intake on maximal isometric knee-extension torque during immobilization and rehabilitation. Values are mean ± S.E.M. of 10 observations and represent the change of maximal isometric knee-extension torque compared with baseline value which was set equal to zero. S.E.M.'s of some data points are omitted for clarity of the figure. A cast first immobilized the right leg (□,□) during a period of 2 weeks, while the other leg served as a

control leg (○,●). Thereafter subjects participated in a 10 weeks rehabilitation program for the knee extensors of the immobilized leg. Subjects ingested either supplementary creatine monohydrate (filled symbols) or placebo (open symbols). * Refers to a significant treatment-effect compared with placebo, $P<0.05$.

**Figure 3**: Effect of oral creatine intake on power output during a bout of maximal dynamic knee-extension exercise during immobilization and rehabilitation. Values are mean ± S.E.M. of 10 observations and represent the change of mean power production during a series of 30 maximal dynamic knee-extensions, compared with baseline value which was set equal to zero. S.E.M.'s of some data points are omitted for clarity of the figure. A cast first immobilized the right leg (□,■) during a period of 2 weeks, while the other leg served as a control leg (○,●). Thereafter subjects participated in a 10 weeks rehabilitation program for the knee extensors of the immobilized leg. Subjects ingested either supplementary creatine monohydrate (filled symbols) or placebo (open symbols). * Refers to a significant treatment-effect compared with placebo, $P<0.05$.

**Figure 4**: Effect of oral creatine intake on relaxation time of quadriceps and hamstring muscles during immobilization and rehabilitation. Values are mean ± S.E.M. of 10 observations and represent the time for muscles to relax from 75% to 25% of the maximal isometric torque. A cast first immobilized the right leg during a period of 2 weeks, while the other leg served as a control leg. Thereafter subjects participated in a 10 weeks rehabilitation program for the knee extensors of the immobilized leg. Subjects ingested either supplementary creatine monohydrate (filled symbols) or placebo (open symbols). Muscle relaxation time was measured in milliseconds (ms) in both hamstring and quadriceps muscles, following a maximal isometric contraction of the respective muscles. The time points on

the x-axis refer to baseline measurements before immobilization (times 1, 6, 11, 16), after immobilization (times 2, 7, 12, 17), after 3 weeks of rehabilitation (times 3, 8, 13, 18) and after 10 weeks of rehabilitation (times 4, 9, 14, 19) for the right and left m. quadriceps and for the right and left hamstrings, respectively. & refers to a significant treatment effect compared with placebo, $p<0.05$.

**Figure 5**: Effect of oral creatine supplementation on muscle fibre cross-sectional areas during leg immobilization and rehabilitation. Values are mean ± S.E.M. of 8 observations and represent cross-sectional areas of type I, type IIa and type IIb muscle fibers, respectively. A cast first immobilized the right leg during a period of 2 weeks. Thereafter subjects participated in a 10 weeks rehabilitation program for the knee-extensors of the immobilized leg. Subjects ingested either supplementary creatine monohydrate (filled bars) or placebo (open bars). Muscle fibers were visualized on transversal microsections of needle biopsy samples of m. vastus lateralis by myofibrillar ATPase staining. * Refers to a significant time-effect compared with the post-immobilization value, $p<0.05$.

**Figure 6**: Effect of long-term creatine intake on muscle glycogen during immobilization and rehabilitation. Values are means ± S.E.M. (n = 8). Before and after two weeks of immobilization and after 3 and 10 weeks of rehabilitation of the right leg a muscle biopsy was taken from the vastus lateralis. During immobilization and rehabilitation subjects ingested creatine monohydrate (closed symbols) or placebo (open symbols). Muscle glycogen was determined by standard enzymatic spectrofotometrical assays. * $p<0.05$ compared with placebo values.

**Figure 7**: Effect of long-term creatine intake on muscle fiber capillarization during immobilization and rehabilitation. Values are means ± S.E.M. (n = 6). Before and after two weeks of immobilization and after 3 and 10

weeks of rehabilitation of the right leg a muscle biopsy was taken from the vastus lateralis. During immobilization and rehabilitation subjects ingested creatine monohydrate (closed symbols) or placebo (open symbols). Muscle capillaries in type I (panel A), type IIa (panel B) and type IIb (panel C) muscle fibers were visualized by PAS analyses.

**Figure 8**: Effect of acute and long-term creatine intake on the response of blood glucose concentration to oral glucose intake. Values are means ± S.E.M. (n = 8-9). Subjects ingested 1g glucose·$kg^{-1}$ BW at time $t_0$ after 12 weeks (panel B) of either oral creatine supplementation (closed symbols) or placebo (open symbols), and 10 weeks following cessation of the creatine or placebo intake (panel A). Thirty min prior to the glucose administration ($t_{-30}$), subjects ingested 10g of creatine monohydrate or placebo. Blood glucose concentration was measured by 15 min intervals on capillary blood samples. * $p<0.05$ compared with the corresponding placebo value.

**EXAMPLES**

**EXAMPLE 1**

Use of creatine for the treatment of disuse atrophy

1. **Materials and Methods**

1.1 Subjects

Twenty-two healthy subjects, 12 males and 7 females, ranging in age from 20 to 23 years gave their informed written consent to take part in the study. They were informed in detail of all experimental procedures to be undertaken and were asked to abstain from any medication during the period of the study and to avoid changes in their diet or level of physical activity. Three of the female subjects were taking oral contraceptive medication.

1.2 Study Protocol

A double-blind study was performed over a 12-week period. During the first week of the study, baseline measurements were performed (Session 1, Week 0). On day 1, and after a light standardized meal (600 kcal, 60% carbohydrates, 25% fat, 15% proteins) m. quadriceps cross-sectional area (CSA) was measured by Magnetic Resonance Imaging (MRI) after which a percutaneous needle biopsy of the right m. vastus lateralis was taken for biochemical and histochemical analyses. On day 4, and again after a light standardized meal, isometric and dynamic maximal knee-extension torque of the right and left leg was evaluated using an isokinetic dynamometer. Subsequently, subjects were assigned to either a creatine (CR: n = 11) or a placebo (P: n = 11) group enabling two groups of similar sex and m. quadriceps cross-sectional area to be obtained. From the next day CR subjects ingested 5g of creatine monohydrate 4 times per day. The creatine supplements were flavored by the addition of citrate ($60mg \cdot g^{-1}$ creatine) and maltodextrine ($940mg \cdot g^{-1}$ creatine), while the P group ingested only maltodextrine containing citrate ($40mg \cdot g^{-1}$ maltodextrine). Creatine and placebo powders were identical in taste and appearance. Furthermore, subjects were instructed to dissolve the supplements in hot water within 1 min before ingestion. The subject's right leg was then immobilized at a knee angle of ~160° by a light polyester cast, reaching from groin to ankle. Subjects received crutches and permanent free access to private transportation services in order to limit loading of the immobilized leg during the immobilization period. A week later the cast was removed and the knee-joint was passively mobilized for 20 min, after which subjects were allowed to take a shower. Immediately after, a new cast was fitted for another week of immobilization.

At the end of the second week of right leg immobilization the cast was removed and post-immobilization measurements were performed (Session 2,

Week 2). Session 2 was identical to session 1, with the exception that on the 1st to 3rd day following removal of the cast subjects participated in a 30 min physiotherapy session (passive mobilization), aimed at restoring normal knee-joint mobility before the assessment of maximal knee extension torque was made on day 4.

Immediately after session 2, a 10-week rehabilitation program was started. Subjects participated in a unilateral training program for the right leg, at a rate of 3 sessions per week. Each training session consisted of 4 series of 12 unilateral knee-extensions ranging from a 90° knee-angle to full extension at a rate of $60° \cdot sec^{-1}$, interspersed by 2 min rest intervals, on a knee-extension apparatus (Technogym®). The workload was set at 60% of maximum isometric knee-extension torque, which was measured at a 90° knee-angle at the start of each session using a calibrated force transducer. During the last 7 weeks of the training period, a series of 6 instead of 4 unilateral knee-extensions were performed. All training sessions were supervised by one of the investigators. The dose of CR or P ingested was reduced from 4 times 5g per day during immobilization to 3 times 5g per day during the initial 3 weeks of rehabilitation, and thereafter to a single 5g daily.

After 3 (Session 3, Week 5) and 10 (Session 4, Week 12) weeks of rehabilitation, and at least 48 hours following the last training session, subjects returned to the laboratory for an intermediate and a post-training evaluation session, respectively. All measurements over the course of sessions 1 to 4 were done on the same day of the week and at the same time of the day for each subject. The results of the measurements were disclosed neither to the subjects nor to the investigators until completion of the study. Determination of m. quadriceps cross-sectional area (CSA)

N.M.R. imaging was performed in a 1.5 T scanner (Vision, Siemens) using a phased array body coil positioned over the upper legs. Subjects lay in supine

position with a plastic leg mould fitted, allowing accurate and reproducible positioning of the subject's legs with reference to the coil and magnet during different MRI sessions. T1 weighted images were acquired with a spin-echo sequence (TR/TE=500/12 ms). First, the most distal point of the medial condylus of the femur was located by frontal scanning. Thereafter, three axial slices with 10 mm thickness and 30 mm spacing, were positioned on coronal slices at 17, 20 and 23 cm proximal to the reference point. The in-plane resolution of the axial images was 1.12 x 0.78 mm. Images were transferred to a dedicated workstation for quantitative processing. CSA of m. quadriceps was determined by digitisation of the images using Visual Basic software (Microsoft Co., USA). Digitisation was done in duplicate by two independent investigators, after which values were averaged. However, if either intra-observer or inter-observer differences for a given image exceeded 5%, the investigators re-digitised the image. For each single image, CSA was eventually calculated as the mean of the values obtained by the two investigators. Finally, quadriceps CSA ($cm^2$) was defined as the mean of the 3 axial scan images taken at 17, 20 and 23 cm proximal to the medial condylus of the femur, respectively.

Determination of knee-extension torque

Maximal voluntary torque and fatigue, and relaxation time of the knee-extensors was evaluated on a self-constructed isokinetic dynamometer that was calibrated prior to each experiment. The dynamometer consisted of a computer controlled asynchronous electromotor (AMK Dynasyn, 19kW), instrumented with a torque transducer (Lebow, maximal torque 565 Nm, 0.05% precision). Prior to the study, subjects reported twice to the laboratory to be familiarized with the test procedure. The exercise test consisted of unilateral knee-extensions performed in a sitting position on the dynamometer. After a 5-min standardized warm-up, the subjects performed 3 voluntary maximal isometric

contractions (3 s), interspersed by 2-min rest intervals, at a knee-angle of 110°. Maximal isometric torque (Nm), and relaxation time (msec) was then obtained from the smoothed curve of the static torque. Relaxation time was defined as the time of torque decay from 75% to 25% of the maximal isometric torque. On the next day, and again after a standardized 5-min warm-up, subjects performed a bout of 30 dynamic maximal voluntary knee-extensions at a constant velocity of $180° \cdot sec^{-1}$, starting from 90° to full extension (180°). After each contraction, the leg was returned ($180° \cdot sec^{-1}$) passively to the starting position from which the next contraction was immediately initiated. Torque and angular velocity were measured during each contraction and were simultaneously digitised (250 Hz) by an on-line computer. Finally, power was calculated from the registered torque and velocity measurements.

1.3 Muscle biochemistry and histochemistry

Muscle samples were obtained from the m. vastus lateralis of the right leg, using the needle biopsy technique (Bergström, J. Scandinavian Journal of Clinical and Laboratory Investigation 14 (suppl. 68), 1-110. 1962) with suction being applied. Incisions were made through the skin and muscle fascia following the administration of local anaesthesia (2-3 ml of 1% lidocaine). During sessions 2, 3 and 4, the incision was made either proximal or distal to the incision made at an earlier session. Following removal from the limb, a piece of each muscle biopsy was immediately freed from blood and visible connective tissue, rapidly frozen in liquid nitrogen, and stored at -80°C for subsequent biochemical analysis. The remaining muscle was mounted in embedding medium, frozen in isopentane, cooled to its freezing point in liquid nitrogen, and stored at -80°C until analyses were performed at a later date. For muscle substrate and enzyme assays muscle samples were freeze-dried and washed twice in petroleum ether to remove fat.

Thereafter, a portion of each sample was dissected free of visible blood and connective tissue and was pulverized. Part of the powdered extract (3-5 mg) was then used for spectrophotometric determination of ATP, PCr and free Cr concentrations (Harris, R. et al., Scandinavian Journal of Clinical and Laboratory Investigation 33, 109-120. 1974). Muscle total creatine concentration was calculated as the sum of PCr and free Cr concentrations. For the histochemical analyses, serial transverse sections (10$\mu$m) were cut from the biopsies with a microtome at -20°C and stained for myofibrillar ATPase to identify fibre types (Brooke, M.H. and Kaiser, K.K. Journal of Histochemistry and Cytochemistry 18, 670-672. 1970).

1.4 Statistical analysis

All data are expressed as mean ± S.E.M. Statistical evaluation (Statistica® software, Ohio, USA) of the data was performed using repeated measures 2-way analysis of variance, using Tuckey's test for post-hoc multiple comparisons where appropriate. The relationship between variables was calculated by Pearson's correlation coefficient. The level of statistical significance was set at $p<0.05$.

2. **Results**

2.1 Body mass and treatment identification

At the start of the study body mass was 66.9 ± 2.7 kg in P versus 65.9 ± 3.1 kg in CR. Over the course of the study, body mass increased ($p<0.05$) in both groups, but there was no significant difference between P and CR at any time during the study. Body mass in P was 68.2 ± 2.8, 68.1 ± 2.8 and 68.4 ± 3.0 kg immediately after immobilization and after 3 and 10 weeks of rehabilitation, respectively. Corresponding values in CR were 66.7 ± 3.2, 67.3 ± 3.2 and 68.4 ± 3.3 kg. The training workload during rehabilitation was similar for

the 2 groups over the entire rehabilitation period. Initial knee-extension 1RM was 67 ± 9 kg in P versus 70 ± 8 kg in CR, and increased to 103 ± 10 kg and 98 ± 10, respectively, during the final stage of the rehabilitation period. At the end of the study subjects were asked whether they had any notion of the treatment they had received but, irrespective of the supplement received, all were unsure. No side effects were reported during the entire duration of the study.

2.2 Muscle cross-sectional area

2.2.1 Immobilized leg

At baseline, m. quadriceps cross-sectional area (CSA) was 90 ± 5 $cm^2$ in P versus 92 ± 5 $cm^2$ in CR. As shown in Figure 1, 2 weeks of immobilization decreased m. quadriceps CSA by ~10% ($p<0.05$, range: 0-15%) to 81 ± 4 in P and 82 ± 5 $cm^2$ in CR. During subsequent rehabilitation, m. quadriceps CSA increased at a faster rate in CR than in P ($p<0.05$). Compared with post-immobilization, after 3 and 10 weeks of rehabilitation in CR m. quadriceps CSA increased by 15% (range: 9-19%) and 21%(range: 20-26%), respectively. However, in P, the corresponding increases amounted to only 9% and 14%. Thus, compared with pre-immobilization baseline values, at the end of the 10-week rehabilitation period the right m. quadriceps CSA was greater ($p<0.05$) in CR (100 ± 6 $cm^2$) but not in P (93 ± 4 $cm^2$).

2.2.2 Control leg

At the start of the study quadriceps CSA was 91 ± 4 $cm^2$ in P and was similar in CR (93 ± 5 $cm^2$). In P, m. quadriceps CSA did not significantly change throughout the study (Figure 1). Conversely, in CR m. quadriceps CSA progressively increased to a value that was higher ($p<0.05$) than at baseline at week 12 (100 ± 6 $cm^2$).

2.3 Muscle strength

2.3.1 Immobilized leg

At the start of the study, maximal isometric knee-extension torque was 151 ± 12 in P and 141 ± 10 Nm in CR. As shown in Figure 2, immobilization decreased torque to the same degree (~27%, range: 0-31%) in both groups (-34 ± 6 Nm in P Vs -28 ± 5 Nm in CR). However, during subsequent rehabilitation, torque increased at a faster rate in CR than in P ($p<0.05$). In CR, after 3 and 10 weeks of leg extension training, maximal isometric torque was increased by 38% (range: 16-56%) and 50% (range: 15-65%), respectively. Corresponding increments in P amounted to 32% (range: 20-40%) and 42% (range: 24-52%). Thus, compared with the pre-immobilization baseline, at the end of the rehabilitation period muscle torque had increased ($p<0.05$) by 10% and 20% in P and CR, respectively. Mean power production during 30 maximal dynamic knee-extensions at baseline was 152 ± 16 watt in CR versus 160 ± 15 watt in P (n.s., see Figure 3). Immobilization decreased ($p<0.05$) power to the same degree (~25%) in both groups (CR, 113 ± 12 watt, P, 122 ± 12 watt). However, during rehabilitation, power output increased at a faster rate in CR than in P ($p<0.05$). Compared with the pre-immobilization baseline, at the end of the 10-week rehabilitation period the mean power production during the bout of maximal dynamic knee-extensions tended to be higher (+13%, n.s.) in CR (172 ± 16 watt) and similar in P (165 ± 17 watt).

2.3.2 Control leg

Maximal isometric knee-extension torque of the control leg was unchanged in both groups during immobilization (Figure 2), but increased during rehabilitation ($p<0.05$). Maximal torque also increased more ($p<0.05$) in CR than in P during the rehabilitation period. Thus, after 12 weeks of creatine supplementation, maximal torque (159 ± 12 Nm) was on average 10% higher in CR than in P ($p<0.05$). Power production during the

dynamic knee-extension exercise was 144 ± 14 watt and 153 ± 15 watt at baseline in CR and P, respectively (Figure 3). In P, power was stable around ~155 watt throughout the study, whereas in CR it progressively increased (+14%, $p<0.05$) to peak at 163 ± 16 watt at the end of the 12-week creatine supplementation period.

2.4 Muscle relaxation time

Relaxation time was measured in quadriceps and hamstring muscles of both the immobilized leg (right leg) and the control leg (left leg) following maximal isometric contraction. As shown in Figure 4, at baseline relaxation times were on the average ~75-80 msec for the m.quadriceps and ~80-90 msec for hamstrings in both P and CR. During P muscle relaxation time did not significantly change over the 12-week treatment period, either in the right leg during immobilization and rehabilitation, or in the left leg. Conversely, creatine administration caused muscle relaxation times to significantly decrease. Thus, whilst muscle relaxation time tended to increase in P during immobilization in both quadriceps and hamstring muscle (n.s.), it decreased ($p<0.05$) in CR. Accordingly, after 3 and 10 weeks of rehabilitation relaxation times were markedly lower ($p<0.05$) in CR than in P, both in the right and in the left leg and in both the quadriceps muscle and the hamstrings.

2.5 Muscle histochemistry

Due to the labor intensive nature of the analysis, histochemical measurement of biopsy material of only the immobilized leg was performed in a subgroup of 16 subjects. At baseline, absolute muscle fibre cross sectional area ($CSA_f$; see Figure 5) in type I, type IIa and type IIb fibers was not significantly different between groups. During immobilization $CSA_f$ did not change significantly in any fibre type in P or CR. Compared with post-immobilization values, during the 10-week rehabilitation period $CSA_f$ in P increased ($p<0.05$) on the

average by 20%, 30% and 37% in type I, type IIa, and type IIb fibers, respectively. Corresponding increments were, on average, greater in CR (I +37%; IIa +59%; IIb +56%). However, due to large data variability, in conjunction with the small number of observations, a statistically significant difference between groups was not achieved. In type I fibers, $CSA_f$ expressed relative to total CSA ($\%CSA_f$), from baseline to the end of the rehabilitation period decreased ($p<0.05$) from 60±5% to 53±5% in P (n.s.) and from 53±3% to 43±4% in CR. Type IIa $\%CSA_f$ concomitantly increased ($p<0.05$) from 29± 3% to 39±5% in CR. Corresponding values in P were 28±4% and 34±3% (n.s.). Type IIb $\%CSA_f$ was stable throughout the study at ~13% in P versus ~17% in CR.

2.6 Muscle biochemistry

Muscle phosphocreatine (PCr) concentration at baseline was not significantly different between the 2 groups (table 1). During immobilization, PCr concentration decreased ($p<0.05$) to about 15% below baseline in P. This fall was completely prevented by creatine supplementation ($p<0.05$). In P, the muscle PCr concentration returned to pre-immobilization baseline value within the initial 3 weeks of rehabilitation, after which the level was maintained. Conversely, in CR, the muscle PCr concentration increased ($p<0.05$) to ~12% above the baseline value after 3 weeks of rehabilitation. However, this PCr "overshoot" was reversed during the final stage of the rehabilitation period.

The muscle free creatine concentration was similar in P and CR throughout the study. However, compared with the pre-immobilization baseline value, muscle free creatine concentrations were higher after both immobilization and rehabilitation ($p<0.05$). Muscle total creatine concentration at baseline was similar in P and CR. In P, immobilization did not change total creatine level. However after 3 weeks but not 10 weeks of rehabilitation it was slightly increased ($p<0.05$).

Conversely in CR, muscle total creatine was higher after immobilization period and following the initial 3 weeks of knee-extension training compared with P. However, together with the declining muscle PCr (table 1), muscle total creatine concentration had returned to the baseline value by the end of the rehabilitation schedule. Muscle ATP concentration ranged from 17.5 ± 0.6 to 21.5 ± 0.5 mmol·kg$^{-1}$ D.W. and was not significantly affected by immobilization or rehabilitation in either experimental group.

Table 1

| | Immobilization | | Rehabilitation | |
|---|---|---|---|---|
| | pre | post | 3 weeks | 10 weeks |
| Free creatine | | | | |
| Placebo | 31.3±3.3 | 41.3±3.6† | 43.5±5.4† | 37.7±2.9† |
| Creatine | 30.6±2.9 | 48.5±4.5† | 53.9±5.4† | 43.4±4.0† |
| Phosphocreatine | | | | |
| Placebo | 76.5±1.8 | 64.9±3.1 †* | 73.8±2.6 | 71.6±2.2† |
| Creatine | 82.4±6.2 | 80.2±5.8 | 89.7±6.8* | 75.1±6.3† |
| Total creatine | | | | |
| Placebo | 108.8±2.8 | 106.2±5.7 | 117.3±5.1† | 109.3±3.4 |
| Creatine | 113.9±8.4 | 128.7±11.6†* | 143.6±11.6†* | 118.5±8.0 |

Values are mean ±S.E.M.of 8 observations and represent concentrations ($\mu$mol.g$^{-1}$ DW) measured in needle biopsy samples obtained from m.vastus lateralis. Total creatine concentration was calculated as the sum of free creatine and phosphocreatine concentrations measured. A cast first immobilised the right leg during a period of 2 weeks. Thereafter subjects participated in a 10-week rehabilitation program for the knee-extensors of the immobilised leg. Subjects ingested either supplementary creatine monohydrate or placebo. See METHODS for further details.

* refers to a significant treatment-effect compared with placebo, $p<0.05$. † refers to a significant time-effect compared with the pre-immobilisation value, $p<0.05$.

**EXAMPLE 2**

Effect of oral creatine supplementation on muscle glycogen and glucose tolerance during immobilization and rehabilitation

1. **Methods**

1.1 Subjects

Eight male (21.7 ± 0.3 yr, 71.5 ± 2.9 kg) and nine female (21.7 ± 0.4 yr, 64.8 ± 1.8 kg) healthy volunteers participated in the study. They gave written consent after having been informed in detail of all the experimental procedures to be undertaken. Subjects were instructed to abstain from any medication and to avoid changes in their usual physical activity level and other living habits during the period of the study. Three of the female subjects were taking oral contraceptives throughout the study. The local ethics committee approved the study protocol.

1.2 Study protocol

At the start of the study 22 subjects were assigned to either a creatine (N=11; CR) or a placebo group (N=11; P) so as to obtain 2 groups of similar distribution for body weight and gender. After baseline measurements, a cast immobilized the subjects' right leg for 2 weeks, where after they underwent a standardized 10-week resistance-training program. The training consisted of 4 to 6 series of 12 unilateral knee-extensions at a workload of 60% of 1RM and at a rate of 3 training sessions per week. During immobilization the CR group received 5g of creatine monohydrate, 4 times per day, while the P group received placebo supplements (5g maltodextrine 4 times per day). During the training period, the creatine/placebo supplements were reduced to 5g 3 times per day from week 1 to 3, and further to a single 5g daily intake from week 4 to 10. The subjects were instructed to dissolve the creatine powders in hot

water immediately before ingestion. The placebo and the creatine supplements were flavored by the addition of citrate and maltodextrine to be identical in appearance and taste. Before (PRE) and after (POST) two weeks of immobilization, and after 3 (R3) and 10 (R10) weeks of rehabilitation a percutaneous needle biopsy of the right vastus lateralis muscle by using the needle biopsy technique with suction being applied. Incisions were made through the skin and muscle fascia under local anesthetic (2-3 ml of 1% lidocaine). During sessions 2, 3 and 4 the incision was made either proximal or lateral to the incision made at an earlier session. A piece of each muscle biopsy was immediately blotted and cleaned from visible connective tissue, rapidly frozen in liquid nitrogen, and stored at -80°C for subsequent biochemical analyses. The remaining muscle was mounted in embedding medium, frozen in isopentane, cooled to its freezing point in liquid nitrogen, and stored at -80°C until histochemically analyzed at a later date.

1.3 Oral glucose tolerance test

Seventeen (CR = 9; P = 8) subjects participated in an oral glucose tolerance test. At the end of the 10-week rehabilitation period, and at least 48 hours following the last training session, the subjects reported to the laboratory in the morning, after an overnight fast. Subjects were seated in a comfortable chair and remained in the seated position during the entire experiment. After 15 min of rest a 75µl capillary blood sample from the hyperemic (Finalgon®) earlobe into Na-heparinized glass capillaries, and a 10ml blood sample from an antecubital vein into Na-heparinized tubes (Vacutainer®), were taken. Immediately after ($t_{-30}$) subjects from the CR group ingested 10g of creatine monohydrate which was dissolved in 150 ml of warm tea, whereas P subjects received the tea only. Thirty min later ($t_0$) a second capillary blood sample was taken, whereupon the subjects ingested 1g of glucose$\cdot kg^{-1}$ BW,

which was dissolved in 300ml of water. Thereafter, additional capillary blood samples were taken at 15 min intervals ($t_{15}$ $t_{30}$, $t_{45}$, $t_{60}$). In addition at $t_{30}$ another 10ml blood sample was taken from an antecubital vein.

Throughout this example data obtained during this experimental session are referred to as the "creatine loaded condition".

After this glucose tolerance test creatine and placebo supplementation was stopped for 10 weeks. Such time period previously has been demonstrated to be largely sufficient to establish a complete wash out of "extra" muscle creatine stores following long-term creatine intake. Thereafter subjects reported back to the laboratory on the same day of the week and time of the day, and underwent an identical oral glucose tolerance test.

1.4 Biochemical and histochemical analyses

Muscle samples for biochemical determinations were first freeze-dried and washed twice in petroleum ether to remove fat. Thereafter a portion of each sample was dissected free of visible blood and connective tissue and was pulverized. The powdered extract was then used for spectrophotometric determination of glycogen, free creatine and phosphocreatine concentration (Harris, R.C. et al., Scand. J. Clin. Lab. Invest. 33: 109-120, 1974).

For the histochemical measurements, serial transverse sections (10$\mu$m) were cut with a microtome at -20°C and stained for myofibrillar ATPase to identify fiber types (Brooke, M.H. and K.K. Kaiser, Journal of Histochemistry and Cytochemistry 18: 670-672, 1970) and with the amylase-periodic acid-Schiff reagent (PAS) method to visualize capillaries (Andersen, P. Acta. Physiol. Scand. 95: 203-205, 1975). Fiber area and capillary density were calculated (Andersen, P. and J. Henriksson, J. Physiol. 270: 677-691, 1977).

Capillary blood samples were immediately analyzed for glucose concentration using an automated glucose analyzer (Y.S.I. glucose analyzer, model 2300 STAT). Thereafter, plasma was separated immediately by high-speed centrifugation and was stored at -80°C until assayed for creatine and insulin concentration.

Plasma creatine was measured using a standard enzymatic fluorometric assay (Bergmeyer, H.U. Methods of Enzymatic Analysis. Weinheim: VCH Verlagsgesellschaft, 1985).

Insulin was determined by a double-antibody radioimmunoassay with rat insulin as the standard (Novo Research Institute, Bagsvaerd, Denmark).

1.5 Data analysis

Muscle total creatine concentration was calculated as the sum of free creatine and phosphocreatine. Capillary diffusional index was calculated by dividing mean muscle fiber area by the number of capillaries per fiber type. Blood glucose disposal was defined as the area under the Δblood-glucose curve (see Figure 8) between 0 and 60 min after glucose ingestion. The glucose area (GA) was calculated according to the trapezoidal rule. All data are given as mean ± SEM.

Differences between experimental groups were statistically evaluated by a repeated measures two-way-analysis of variance using Statistica® software (Statsoft Inc., Tulsa, USA). A probability level of $p<0.05$ was set as the criterion for statistical significance.

## 2. Results

### 2.1 Muscle glycogen

Initial muscle glycogen concentration was 407 ± 43 $\mu mol \cdot g^{-1}$ DW in controls versus 379 ± 19 $\mu mol \cdot g^{-1}$ DW in the creatine group. As shown in Figure 6 muscle glycogen did not significantly change during immobilization in

either group. During the initial 3 weeks of rehabilitation muscle glycogen increased ($p<0.05$) in both groups. However, the increase was greater in CR than in P. Hence after 3 weeks of rehabilitation training, muscle glycogen concentration was higher ($p<0.05$) in CR (660 ± 70 $mmol \cdot kg^{-1}$ DW than in P (520 ± 60 $mmol \cdot kg^{-1}$ DW). However, during the last 7 weeks of the rehabilitation period, muscle glycogen dropped back to the similar baseline values in both groups.

2.2 Histochemistry

In the total group of subjects at baseline the degree of capillarization was highest in type I and type IIa fibers (5.0 ± 0.2 capillaries/fiber in type I vs. 5.1 ± 0.2 in type IIa), and was lower ($p<0.05$) in type IIb fibers (4.4 ± 0.3). However, compared with P (I: 5.4 ± 0.2; IIa: 5.5 ± 0.2; IIb: 4.7 ± 0.3), the number of capillaries in IIa fibers, but not in type I and IIb fibers, was significantly lower ($p<0.05$) in CR (I: 4.5 ± 0.2; IIa: 4.6 ± 0.3; IIb: 3.9 ± 0.4; see Figure 7). The number of capillaries significantly changed in any fiber type during neither immobilization nor during the initial 3 weeks of the rehabilitation period. However, between 3 and 10 weeks of rehabilitation, the number of capillaries increased in CR in type I (n.s.), type IIa ($p=0.09$) and type IIb fibers ($p<0.05$), whilst it was unchanged in P. Thus, at the end of the study, in CR the number of capillaries of all fiber types was ~20-25% higher than at baseline.

Diffusional area indices (DI) at baseline were 10-15% higher in CR than in P. Neither the immobilization, nor the initial 3 weeks of rehabilitation significantly changed DI. However, compared with post-immobilization values, 10 weeks of rehabilitation in P increased (n.s.) DI by about 30% in all fiber types. Conversely, in CR only DI for type IIb fibers increased (+15%, n.s.) during rehabilitation, whilst DI for type I and IIa fibers was unaffected.

2.3 Glucose tolerance test

2.3.1 Plasma creatine.

Plasma creatine concentration in P was constant at 45-80 $\mu mol \cdot l^{-1}$ throughout the glucose tolerance test, both at baseline and after 12 weeks of placebo intake (table 2). In CR, baseline plasma creatine concentration was 62 ± 20 $\mu mol \cdot l^{-1}$ at $t_{-30'}$ and increased ($p<0.05$) to 1.40 ± 0.18 $mmol \cdot l^{-1}$ by 1 hour ($t_{30'}$) after the creatine intake. Corresponding values after 12 weeks of oral creatine supplementation were 111 ± 18 at $t_{-30'}$ versus 1.13 ± 0.12 $mmol \cdot l^{-1}$ at $t_{30'}$, and were lower ($p<0.05$) than at baseline.

Table 2

| | BASELINE | | CREATINE LOADED | |
|---|---|---|---|---|
| | Before | After | Before | After |
| Insulin ($ng.ml^{-1}$) | | | | |
| Placebo | 0.7±0.1 | 2.9±0.5 | 0.7±0.1 | 3.1±0.7 |
| Creatine | 0.7±0.1 | 3.0±0.4 | 0.6±0.1 | 3.2±0.5 |
| Plasma creatine ($\mu mol.l^{-1}$) | | | | |
| Placebo | 47±14 | 65±18 | 66±13 | 78±10 |
| Creatine | 62±20 | 1403±185* | 111±18* | 1131±122* |

Values are means ±S.E.M.of 8-9 observations. Insulin, growth hormone (GH), insulin-like-growyh-factor 1 (IGF-1) and creatine were measured on venous plasma 30 min before and 30 min after oral glucose (1 g glucose.$kg^{-1}$ BW) intake in basekline and creatine loaded subjects. Thirty min prior to the glucose administration, subjects ingested 10g of creatine.$H_20$ or placebo. See Methods for further details. *$p<0.05$ compared to corresponding placebo value.

2.3.2 Blood glucose.

Initial ($t_{-30'}$) blood glucose concentration was similar in both groups (4.61 - 4.65 ± 0.1 $mmol \cdot l^{-1}$) and was not affected by the acute intake of 10g of creatine monohydrate or placebo in the respective groups, either at baseline or after 12 weeks of creatine (4.59 ± 0.2 $mmol \cdot l^{-1}$) or placebo (4.46 ± 0.2 $mmol \cdot l^{-1}$) supplementation.

In P at baseline, ingestion of the oral glucose load ($1g \cdot kg^{-1}$) increased blood glucose to a peak value reaching $8.2 \pm 0.4$ $mmol \cdot l^{-1}$ at $t_{30'}$ where after it returned to $6.7 \pm 0.6$ $mmol \cdot l^{-1}$ at $t_{60'}$ (Figure 8, panel a). In CR, except at $t_{15'}$, blood glucose concentration following the glucose intake was lower, and peaked at $7.6 \pm 2$ $mmol \cdot l^{-1}$ at $t_{15'}$ before returning to $5.9 \pm 0.5$ $mmol \cdot l^{-1}$ by $t_{60'}$. Thus, glucose area (GA) was $154 \pm 21$ $mmol \cdot l^{-1} \cdot min$ in P and tended ($p=0.10$) to be lower in CR ($112 \pm 15$ $mmol \cdot l^{-1} \cdot min$). At the end of the 12-week supplementation period, the blood glucose response ($GA=163 \pm 16$ $mmol \cdot l^{-1} \cdot min^{-1}$) to glucose ingestion in P was similar to baseline (Figure 8, panel b). However, in CR blood glucose concentrations and glucose area ($90 \pm 22$ $mmol \cdot l^{-1} \cdot min^{-1}$) were lower ($p<0.05$). Thus, compared with placebo, creatine supplementation tended to reduce GA following ingestion of the oral glucose load ($p=0.10$).

At baseline, plasma insulin was similar in P and CR (Table 2). Glucose intake obviously increased plasma insulin ($p<0.05$). However, the impact of glucose intake on plasma insulin was independent of acute creatine intake or prior long-term creatine loading.

## CLAIMS

1. Use of a creatine compound, in particular creatine or a creatine analogue for the manufacture of a therapeutic preparation for the prevention or treatment of muscle disuse syndrome in a subject.

2. Use as claimed in claim 1, wherein the subject to be treated need not in addition perform a training programme to increase muscle volume and muscle functional capacity.

3. Use as claimed in claim 1 or 2, **characterized in that** the therapeutic preparation is intended to be administered in an amount that leads to a total daily supplementation of 0,5 to 5 g creatine.

4. Use as claimed in claims 1-3, **characterized in that** the muscle disuse syndrome is the result of immobilization, or reduced level of physical activity due to disease, aging, or (mental or physical) handicap.

5. Therapeutic preparation for treating or preventing muscle disuse syndrome, comprising a suitable carrier, diluent or excipient and an effective amount of one or more creatine compounds.

6. Therapeutic preparation according to claim 5, which is a drug.

7. Therapeutic preparation according to claim 5, which is a nutritional supplement.

8. Therapeutic preparation according to claim 5 or 7, which has the form of a food stuff comprising one or more additional creatine compounds.

Figure 1

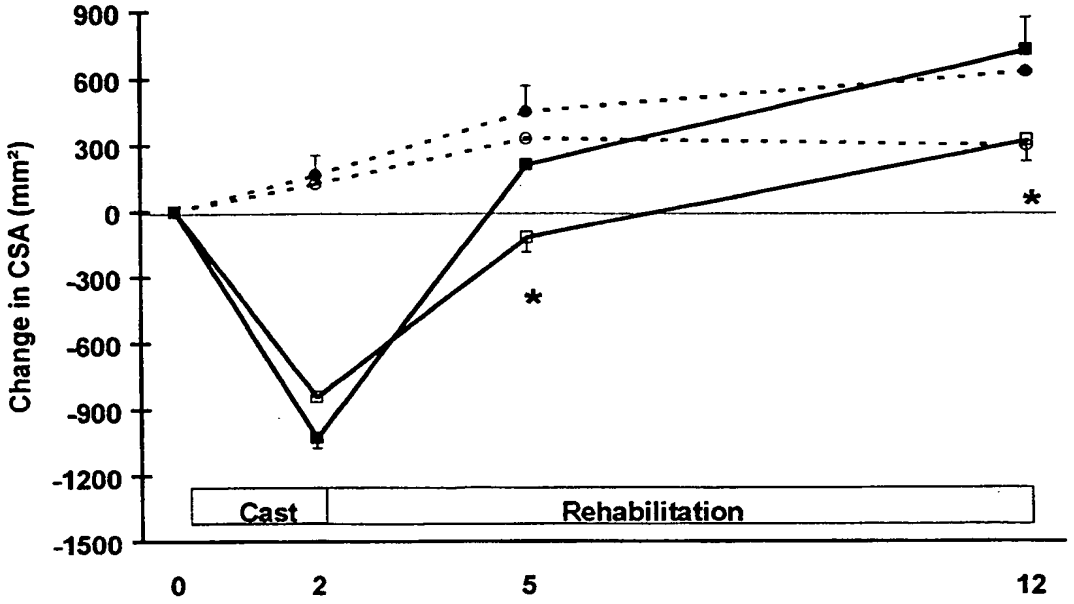
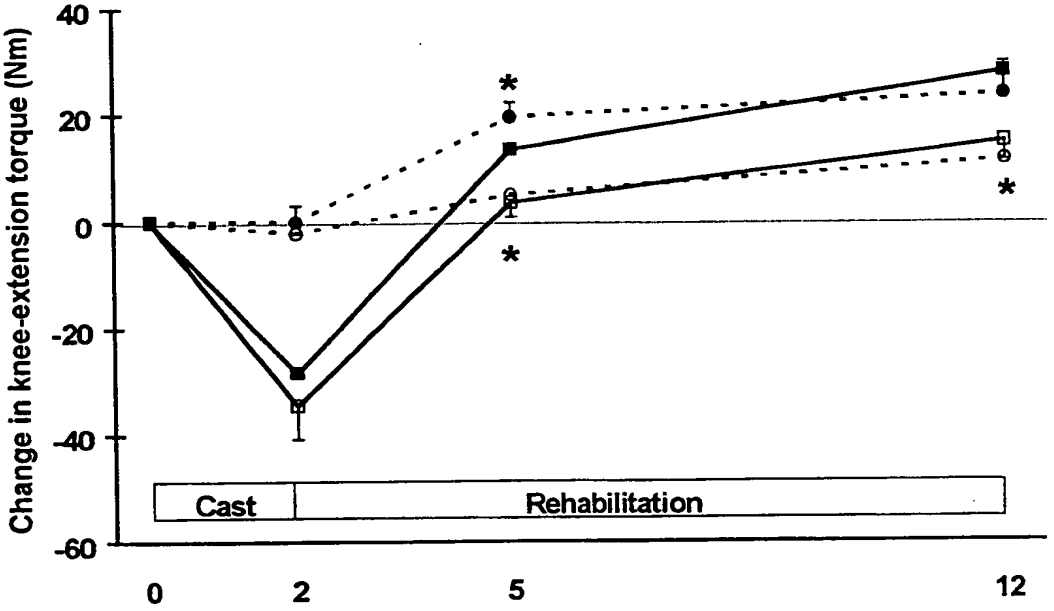
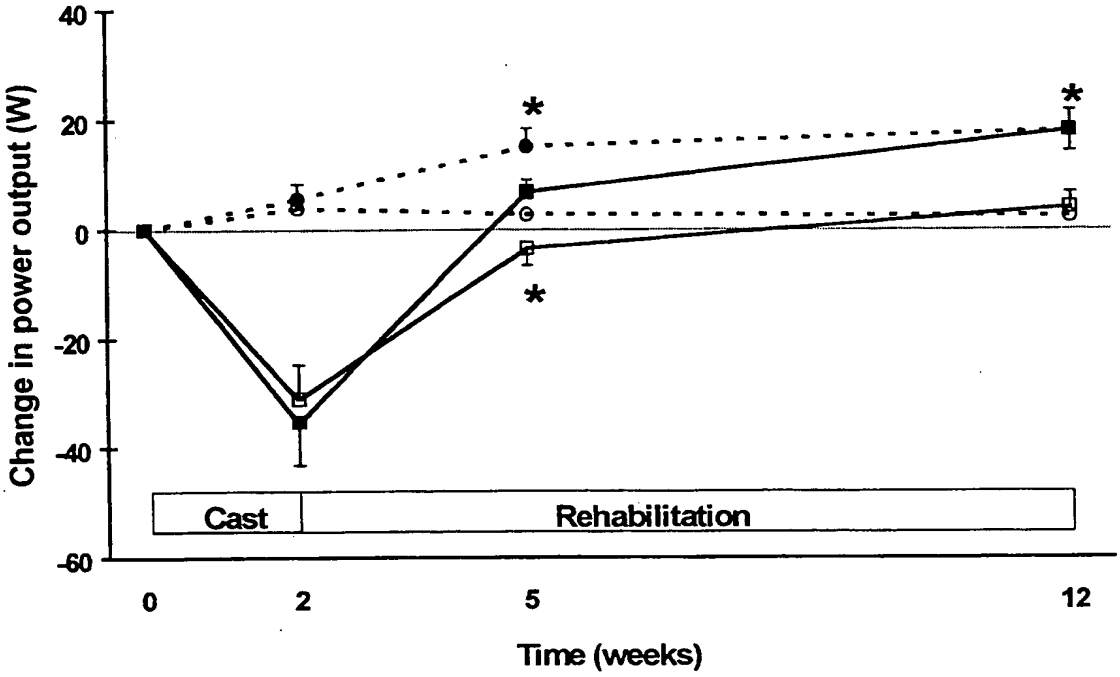

900
600
300
0
-300
-600
-900
-1200
-1500
Change in CSA (mm²)
Cast
Rehabilitation
0
2
5
12
*
*

Figure 2


Change in knee-extension torque (Nm)
40
20
0
-20
-40
-60
*
*
*
Cast
Rehabilitation
0
2
5
12

Figur 3


40
20
0
-20
-40
-60
Change in power output (W)
*
*
*
Cast
Rehabilitation
0
2
5
12
Time (weeks)

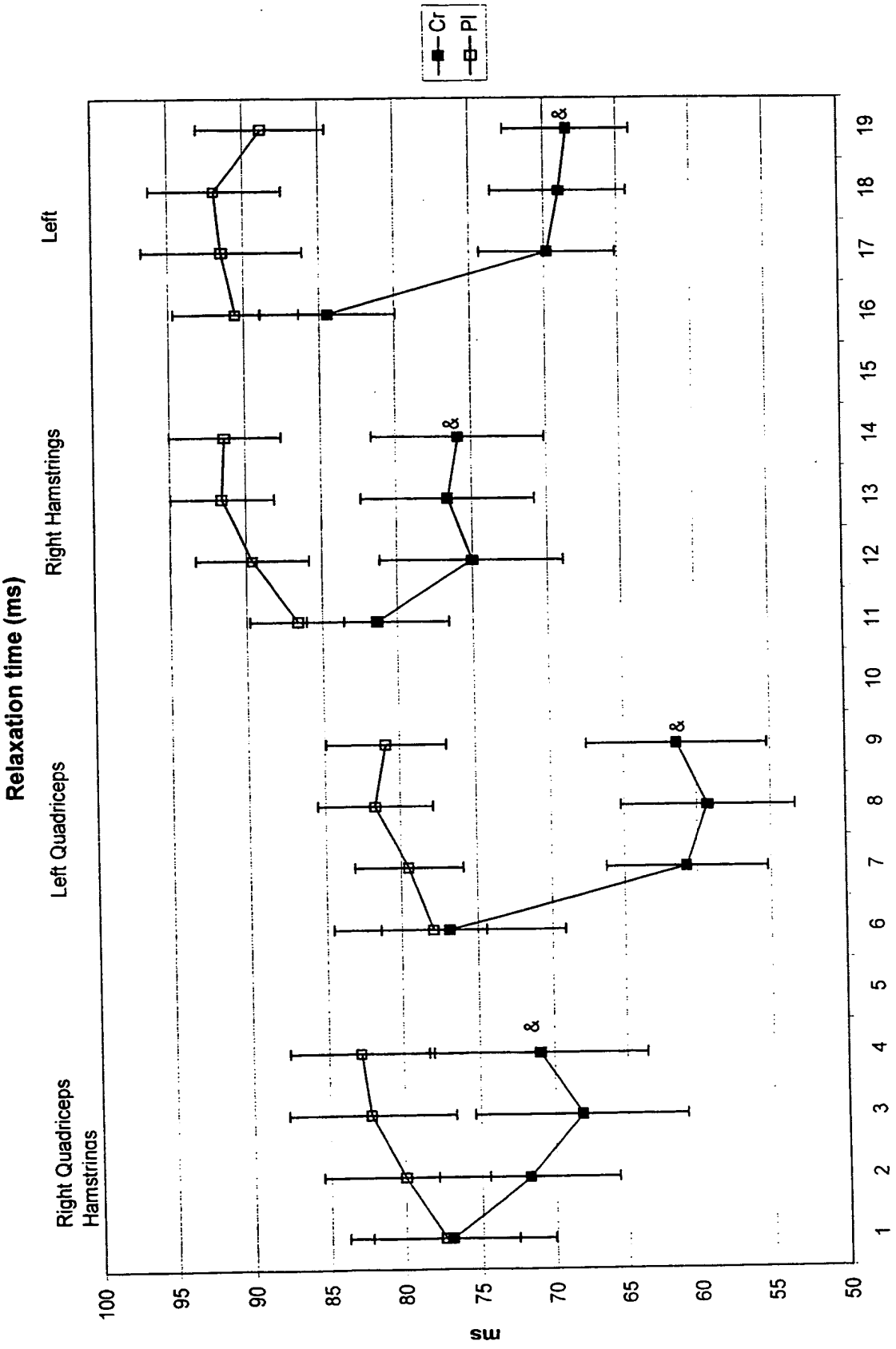
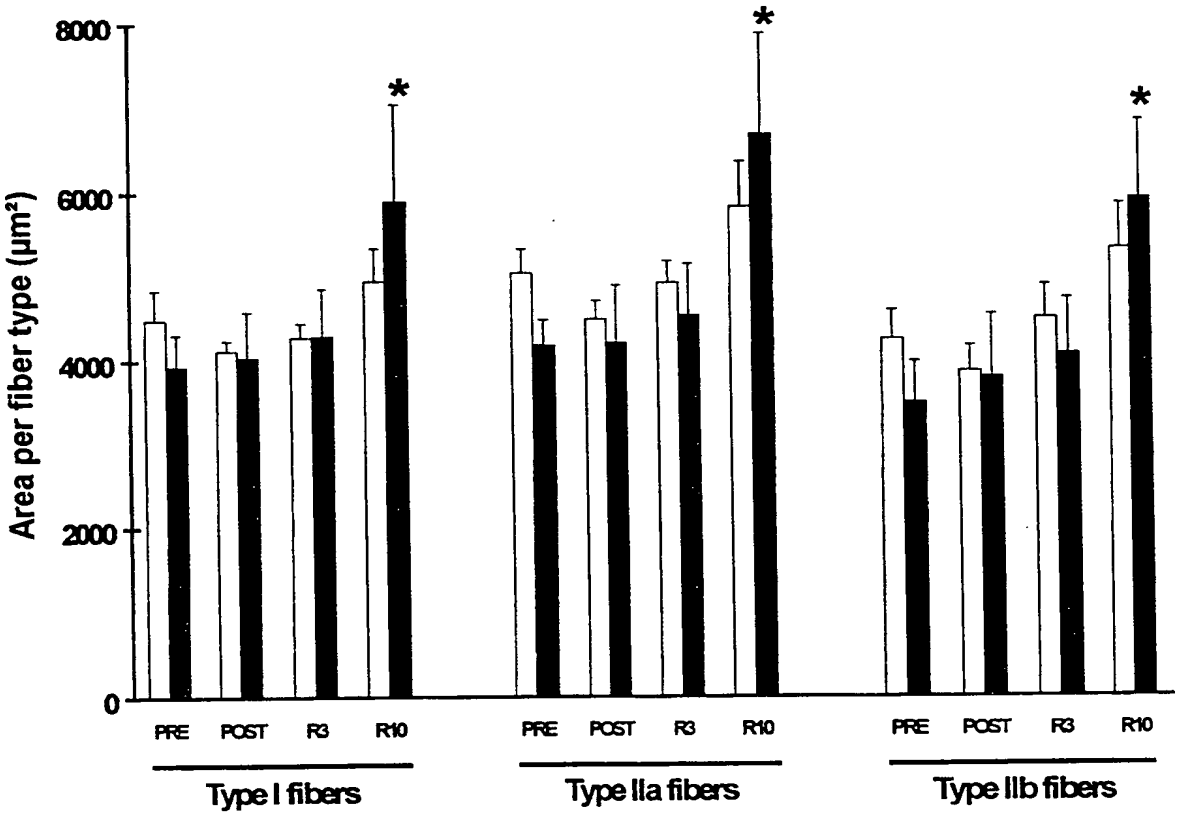

Relaxation time (ms)
Cr
Pl
Left
Right Hamstrings
Left Quadriceps
Right Quadriceps
Hamstrings
ms
100
95
90
85
80
75
70
65
60
55
50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
&

Fig. 4

Figur 5


8000
6000
4000
2000
0
Area per fiber type (µm²)
*
*
*
PRE
POST
R3
R10
PRE
POST
R3
R10
PRE
POST
R3
R10
Type I fibers
Type IIa fibers
Type IIb fibers

Figur 6

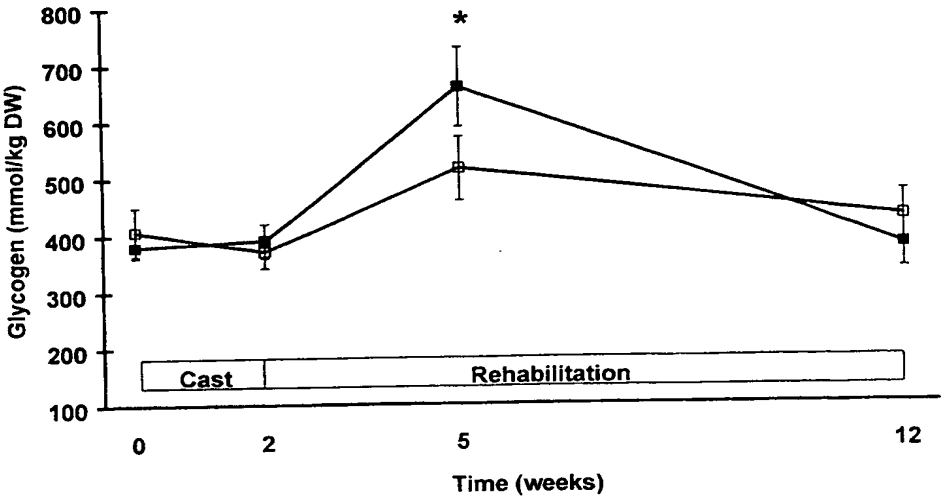

800
700
600
500
400
300
200
100
Glycogen (mmol/kg DW)
*
Cast
Rehabilitation
0
2
5
12
Time (weeks)

Figure 7A

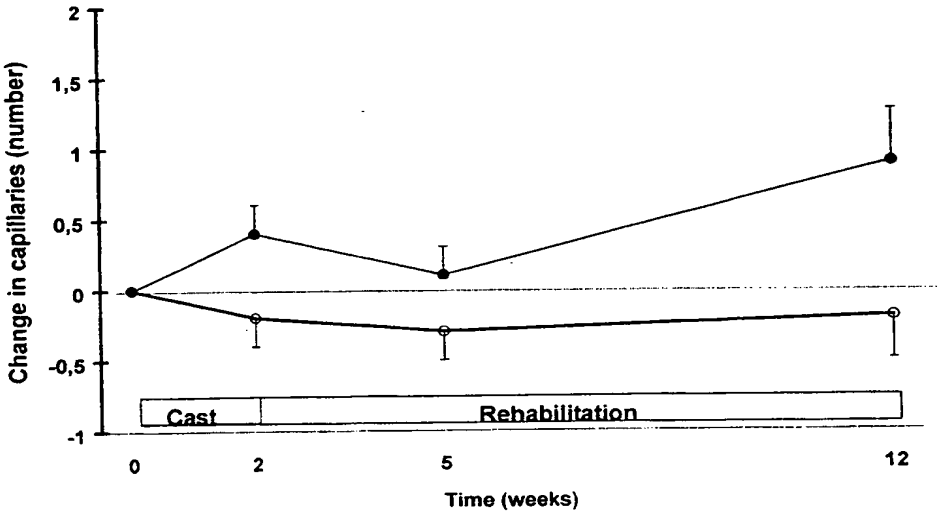
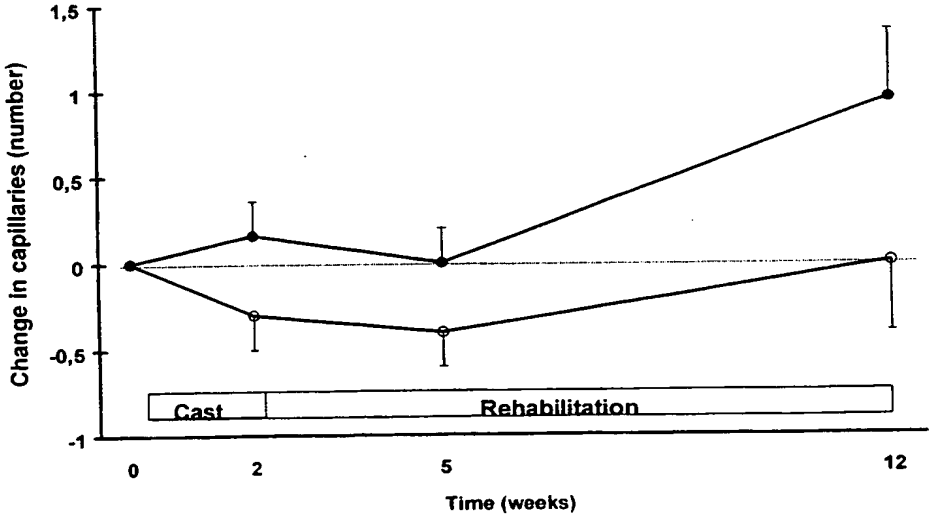



Figure 7B



Figur 7C

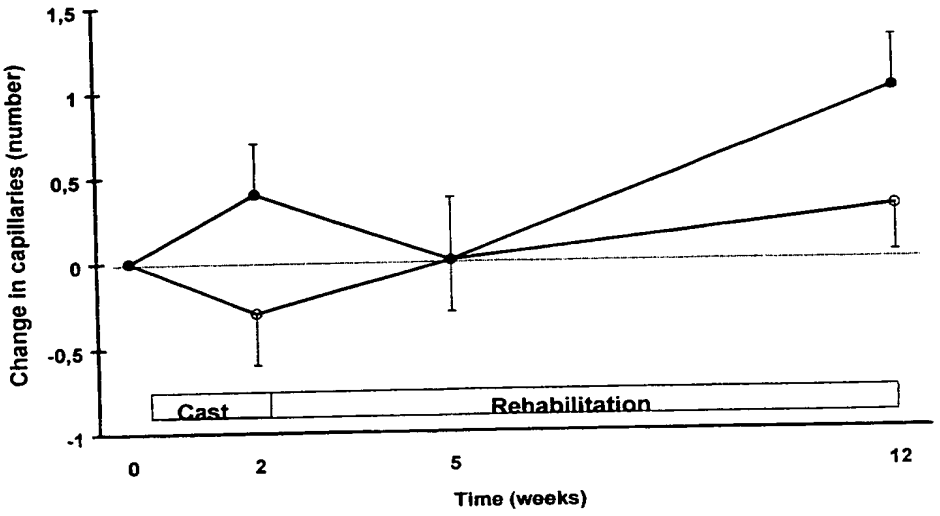

1,5
1
0,5
0
-0,5
-1
Change in capillaries (number)
Cast
Rehabilitation
0
2
5
12
Time (weeks)

Figure 8A

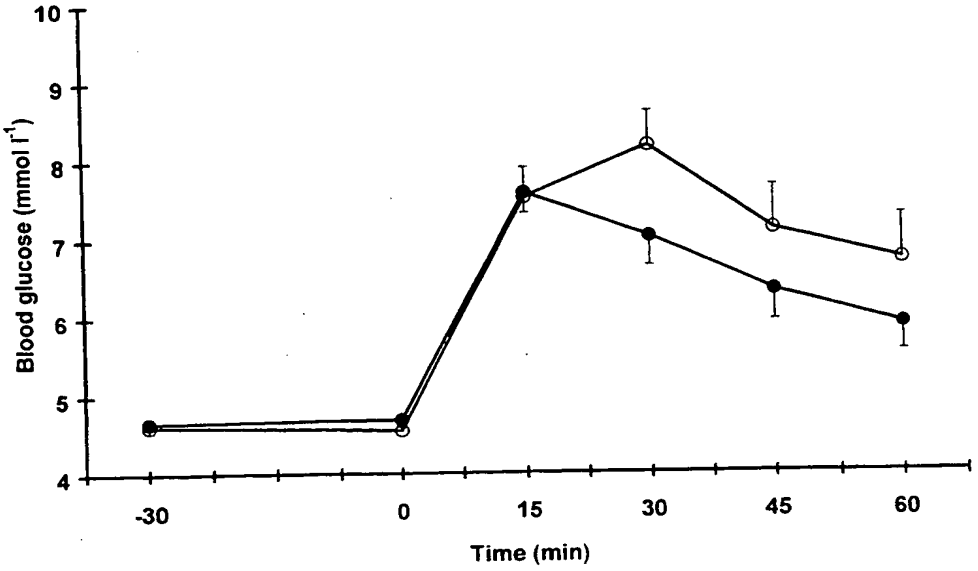
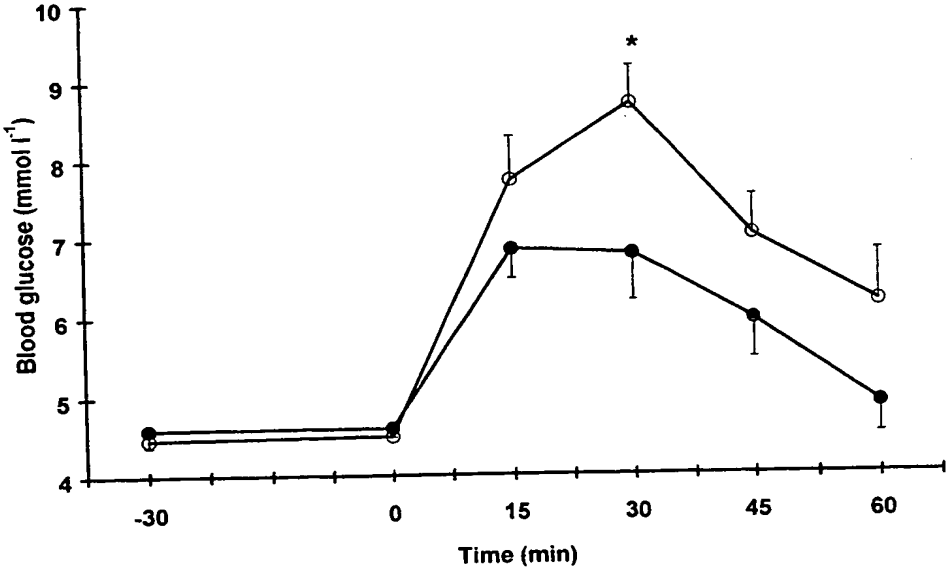



Figure 8B



# INTERNATION SEARCH REPORT

International application No
PCT/EP 99/09137

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WYSS M. ET AL: "The therapeutic potential of oral creatine supplementation in muscle disease." MEDICAL HYPOTHESES, (1998) 51/4 (333-336). REFS: 37 ISSN: 0306-9877 CODEN: MEHYDY, XP002101314 United Kingdom abstract page 335, column 1, paragraphs 2,3 | 1-8 |
| X | MAHANNA, D. A. ET AL: "Effects of.beta.-guanidinopropionic acid on murine skeletal muscle" EXP. NEUROL. (1980), 68(1), 114-21 CODEN: EXNEAC;ISSN: 0014-4886, XP002101313 abstract; table 1 page 120, paragraphs 2,3 | 1-8 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 3 May 2000 | 12/05/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | A. Jakobs |

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | SMITH, SINCLAIR A. ET AL: "Creatine supplementation and age influence muscle metabolism during exercise" J. APPL. PHYSIOL. (1998), 85(4), 1349-1356 CODEN: JAPHEV;ISSN: 8750-7587, XP002101311 abstract page 1350, column 1, paragraph 1 page 1355, column 1, paragraph 3 | 5-8 |
| X | WO 98 00148 A (IPR INST PHARM RES RIEHEN AG ;MEYER HANS (CH)) 8 January 1998 (1998-01-08) abstract page 2, paragraph 3 page 4, line 20 -page 5, line 6; claims 1-10; examples 1-3; tables 1-3 | 1-8 |
| X | BERMON S ET AL: "Effects of creatine monohydrate ingestion in sedentary and weight-trained older adults." ACTA PHYSIOLOGICA SCANDINAVICA, (1998 OCT) 164 (2) 147-55. JOURNAL CODE: 1U4. ISSN: 0001-6772., XP002101316 ENGLAND: United Kingdom abstract | 1-8 |
| X | WO 98 38183 A (GLOXHUBER CHRISTIAN ;SUEDDEUTSCHE KALKSTICKSTOFF (DE); PISCHEL IVO) 3 September 1998 (1998-09-03) abstract page 5, line 26 -page 6, line 9; claims 8,9 | 1-8 |
| X | HEINANEN, K. ET AL: "The effect of long-term creatine and guanidinoacetate supplementation on muscle 31phosphorus spectrum in HOGA." JOURNAL OF INHERITED METABOLIC DISEASE, (1997) VOL. 20, NO. SUPPL. 1, PP. 23. MEETING INFO.: 35TH ANNUAL SYMPOSIUM OF THE SOCIETY FOR THE STUDY OF INBORN ERRORS OF METABOLISM GOTEBORG, SWEDEN SEPTEMBER 2-5, 1997 ISSN: 0141-8955., XP002101312 abstract | 5-8 |
| X | WO 98 43617 A (SOLE MICHAEL J ;JEEJEEBHOY KHURSHEED N (CA)) 8 October 1998 (1998-10-08) abstract page 1, line 4 -page 3, line 2 page 8, paragraph 3 page 13, line 14 -page 14, line 25 | 1-8 |

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | HENRIKSSON, JAN: "Effect of training and nutrition on the development of skeletal muscle." JOURNAL OF SPORTS SCIENCES, (1995) VOL. 13, NO. SPEC. ISSUE, PP. S25-S30. ISSN: 0264-0414., XP002101315 abstract page S28, column 1, paragraph 2 | 5-8 |

1

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/09137

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: –
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark n Protest**

☐ The additional search fees w re accompanied by the applicant's protest.

☐ No protest accompanied the payment f additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 99 /09137

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The Present claims 1-10 relate to a large number of possible compounds. The term creatine analog leads to a lack of clarity within the meaning of Article 6 PCT to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear, namely the compounds for which pharmacological data were given and the compounds specifically mentioned in the claims.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/09137

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9800148 A | 08-01-1998 | AU 3086897 A | 21-01-1998 |
| WO 9838183 A | 03-09-1998 | DE 19707694 A | 27-08-1998 |
| | | AU 6726098 A | 18-09-1998 |
| | | EP 0971911 A | 19-01-2000 |
| | | US 5863939 A | 26-01-1999 |
| | | ZA 9801278 A | 27-08-1998 |
| WO 9843617 A | 08-10-1998 | AU 6715398 A | 22-10-1998 |
| | | EP 0969744 A | 12-01-2000 |

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| ./. | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/EP99/09137 | 19/11/1999 | 19/11/1998 |

International Patent Classification (IPC) or national classification and IPC
A61K31/195

Applicant
K.U. LEUVEN RESEARCH & DEVELOPMENT et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 14/06/2000 | 09.02.2001 |

Name and mailing address of the international preliminary examining authority:

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# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/09137

## I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
**Description, pages:**

1-25 as originally filed

**Claims, No.:**

1-8 as originally filed

**Drawings, sheets:**

1/9-9/9 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | |
| | No: | Claims | 1-8 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-8 |
| Industrial applicability (IA) | Yes: | Claims | 1-8 |
| | No: | Claims | |

2. Citations and explanations
**see separate sheet**

## Comments on item V

1. Reference is made to the following documents:

D1: SMITH, SINCLAIR A. ET AL: 'Creatine supplementation and age influence muscle metabolism during exercise' J. APPL. PHYSIOL.(1998), 85(4), 1349- 1356
D2: WO 98 38183 A, 3 September 1998
D3: WO 98 00148 A, 8 January 1998, cited in the application
D4: WO 98 43617 A, 8 October 1998
D5: HEINANEN, K. ET AL: 'The effect of long-term creatine and guanidinoacetate supplementation on muscle 31phosphorus spectrum in HOGA.' JOURNAL OF INHERITED METABOLIC DISEASE, (1997) VOL. 20, NO. SUPPL. 1, PP. 23
D6: BERMON S ET AL: 'Effects of creatine monohydrate ingestion in sedentary and weight-trained older adults.' ACTA PHYSIOLOGICA SCANDINAVICA, (1998 OCT) 164 (2) 147-55
D7: WYSS M. ET AL: 'The therapeutic potential of oral creatine supplementation in muscle disease' MEDICAL HYPOTHESES, (1998) 51/4 (333-336), cited in the application
D8: HENRIKSSON, JAN: 'Effect of training and nutrition on the development of skeletal muscle.' JOURNAL OF SPORTS SCIENCES, (1995) VOL. 13, NO. SPEC. ISSUE, PP. S25-S30

2. The use of creatine for preparing a composition for preventing or treating muscle disuse syndrome, and particularly due to disease, i.e, the subject-matter of claims 1 and 4, is known from D2 (see the abstract) and D3 (see page 4, lines 20-31). It should be noted that even if the term muscle disuse syndrome is not mentioned in these two documents, they disclose some of the signs which characterize said disorder.

2.1 The additional feature of claim 2 seems to be implicit to the disclosures of D2 and D3, since both documents claim that their creatine-containing preparations are suitable for the therapeutic use previously mentioned. If such preparations alone were not enough, but a training programme was additionally needed, it would be

mentioned in D2 and D3.

2.2 Daily supplementation as in claim 3 are known from D3 (see p.5, l.3-4).

3. Claims 5 to 8 refer simply to a therapeutic preparation containing creatine and a suitable carrier, diluent or excipient, since the wording "...for preventing or treating..." is construed as "suitable for the prevention/treatment of..." (cf. PCT Guidelines, III, 4.8). As creatine-containing preparations are well-known in the art (see sections 3.1 ), and it seems that these known compositions would also be suitable for the claimed use, the subject-matter of claim 5 lacks novelty.

3.1 The additional feature of claim 6 is known from D2 (see p.2, l.27-31), D3 (see p.5, l.8-29) and D5 (see the abstract).

3.2 The additional feature of claim 7 is known from D1 (see p.1350, left column, first paragraph; this document anticipates also the feature of claim 8), D2, D4, D6, D7 and D8 (see the abstracts).